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Regulation of Mitochondrial Protein Turnover by Thyroid Hormone(s)

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1. The effect of thyroidectomy on turnover rates of liver, kidney and brain mitochondrial proteins was examined. 2. In the euthyroid state, liver and kidney mitochondria show a synchronous turnover with all protein components showing more or less identical half-lives compared with the whole mitochondria. The brain mitochondrial proteins show asynchronous turnover, the soluble proteins having shorter half-lives. 3. Mitochondrial DNA (m-DNA) of liver and kidney has half-lives comparable with that of whole mitochondria from these tissues. 4. Thyroidectomy results in increased half-lives of liver and kidney mitochondria, with no apparent change in the half-life of brain mitochondria. 5. A detailed investigation of the turnover rates of several protein components revealed a significant decrease in the turnover rates of mitochondrial insoluble proteins from the three tissues under study. 6. The turnover rates of m-DNA of liver and kidney show a parallel decrease. 7. Thus it is apparent that thyroid hormone(s) may have a regulatory role in maintaining the synchrony of turnover of liver and kidney mitochondria in the euthyroid state. Turnover of brain mitochondria may perhaps be regulated by some other factor(s) in addition to thyroid hormone(s). 8. It seems likely that during mitochondrial turnover m-DNA and insoluble proteins may constitute a major unit. 9. The mitochondrial protein contents of the three tissues are not affected by thyroidectomy. 10. No correlation was seen between the turnover rate of mitochondria and cathepsin activity in any of the tissues under study in normal or thyroidectomized animals. 11. On the other hand, mitochondrial proteinase activity shows good correlation with the turnover rates of mitochondria in normal animals, and a parallel decrease in activity comparable with the decreased rates of turnover is observed after thyroidectomy. 12. It is concluded that mitochondrial proteinase activity may play a significant role in their protein turnover.

Fletcher & Sanadi (1961) first reported that rat liver mitochondria turn over with a half-life of about 10 days. This observation has been confirmed by several investigators (Cuzner *et al.*, 1966; Beattie *et al.*, 1967; Swick *et al.*, 1968; Gross *et al.*, 1969; Menzies & Gold, 1971) and it now seems that mitochondria from tissues such as liver, kidney and brain turn over at a fairly rapid rate.

It is now well recognized that thyroid hormones greatly influence mitochondrial metabolism, thyroidectomy causing decreased protein synthesis and treatment with thyroid hormones resulting in increased metabolism with a higher rate of protein synthesis in these organelles (Freeman *et al.*, 1963; Tata *et al.*, 1963; Gustafsson *et al.*, 1965; Roodyn *et al.*, 1965; Katyare *et al.*, 1970; Satav *et al.*, 1973). It seemed likely therefore that the regulatory role of thyroid hormones may be further elucidated by examining the turnover rates of mitochondrial protein components from different tissues of rats. However, since we had previously observed that during the 10-day period of tri-iodothyronine administration, the turnover of mitochondrial proteins did not follow first-order reaction kinetics (Katyare *et al.*, 1970), the regulatory role of thyroid hormones was ascertained indirectly by using thyroidectomized rats in comparison with normal euthyroid animals. The results of such studies are reported in the present paper and would suggest a tissue-specific regulatory role for the thyroid hormones. Results on mitochondrial protein contents are included. An attempt has also been made to seek a probable correlation between the rates of mitochondrial protein turnover and either lysosomal cathepsin activities or mitochondrial proteinase activities.

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Materials and Methods

Chemicals

All chemicals were of analytical-reagent grade. PPO (2,5-diphenyloxazole) and POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] were purchased from

Sigma Chemical Co., St. Louis, Mo., U.S.A. DL-[1-^{14}C]Leucine (sp. radioactivity 47.9mCi/mmol) and [^{3}H]thymidine (sp. radioactivity 8000mCi/mmol) were obtained from the Isotope Division of this Research Centre. DNAase I (deoxyribonuclease I) (crystallized and freeze-dried) was obtained from Sigma.

Animals

Thyroidectomy was performed on weanling male rats of Wistar strain (20–22 days old, 30–35g body wt.) by surgery as previously described (Katyare *et al.*, 1970; Satav *et al.*, 1973). The animals were allowed to grow for 8–10 weeks; a periodic record of their body weights and basal metabolic rates was kept. Only those animals showing a considerable decrease in body weight (30–35% decrease) and basal metabolic rate (35–40% decrease) were used for further studies. Unoperated littermates of the same age were used as controls.

Isolation of mitochondria

Rats were killed by decapitation and their liver, kidneys and brain were quickly removed and kept in chilled isolation media. Liver and kidney mitochondria were isolated from a 10% (w/v) homogenate made in the isolation medium containing 0.25M-sucrose, 10mM-Tris–HCl, pH7.4, and 1mM-EDTA by using a Potter–Elvehjem-type homogenizer. Liver and kidney mitochondria were isolated by centrifuging nuclei-free homogenates at 6500*g* for 10min (Katyare *et al.*, 1971) and 12000*g* for 10min (Johnson & Lardy, 1967) respectively. Brain mitochondria were isolated as described by Ozawa *et al.* (1966), by using 0.3M-mannitol containing 0.1mM-EDTA, pH7.4, as the isolation medium. All operations were carried out at 0–4°C.

Tissues from two or three normal and three or four thyroidectomized animals were pooled for isolation of mitochondria.

All mitochondria were washed three times with their respective isolation media.

Fractionation of mitochondrial protein components

Mitochondrial protein components, namely water-soluble proteins, cytochrome *c*-containing fraction, contractile proteins, structural proteins and 'other cytochromes' were fractionated essentially as described by Beattie *et al.* (1966).

Isolation of DNA

Mitochondria were incubated with DNAase I to remove extramitochondrial DNA (Gross *et al.*, 1969). Mitochondrial DNA was extracted as described by Parsons & Simpson (1973). DNA was determined by the method of Burton (1956), by using calf thymus DNA as a standard.

Injection of label for turnover studies

[1-^{14}*C*]*Leucine*. The animals received, in 0.9% NaCl solution, 25μCi of DL-[1-^{14}C]leucine/100g body wt. intraperitoneally. The label was allowed to stabilize for 1 week (Fletcher & Sanadi, 1961) and the animals were killed at the end of 1, 2, 3 or 4 weeks.

[^{3}H]*Thymidine*. The animals received two injections of [^{3}H]thymidine (100μCi/100g body wt. each) on 2 consecutive days and were killed on days 1, 7, 15 or 21 after the second injection.

Measurements of radioactivity

The radioactivity in the protein was determined by using a cylinder-plating method as described previously by using a scintillator consisting of 0.3% PPO and 0.01% POPOP in toluene (Katyare *et al.*, 1970; Satav *et al.*, 1973). The counting efficiency for ^{14}C was 95%.

For counting the radioactivity in DNA nucleotides, the scintillator described in the Beckman LS 100 manual (Beckman Instruments, Scientific Instrument Division, Fullerton, Calif., U.S.A.) was used, which consisted of 10% naphthalene and 0.5% PPO in dioxan. The counting efficiency for ^{3}H was 7%. Vials were allowed to stabilize overnight to avoid spurious counts.

Half-lives of the protein components and of DNA were calculated by following the equation of first-order reaction kinetics (Fletcher & Sanadi, 1961).

Enzyme assays

Succinate dehydrogenase activities were determined on tissue homogenates and thrice-washed mitochondria, by the procedure of Caplan & Greenwalt (1968). Specific mitochondrial protein content of the tissues was computed from these activities (Gross, 1971).

Cathepsin activity was assayed as described by Gianetto & de Duve (1955); for determination of total activity, the homogenate was subjected to freezing and thawing six times. In addition, a small amount of Triton X-100 was also included in the system. The amount of Folin-positive material was determined by using tyrosine as a standard (Spies, 1957).

Mitochondrial proteinase activity was assayed by incubating 10mg of mitochondrial proteins in 25mM-sodium phosphate buffer, pH6.0, in a total volume of 4ml. The incubation was carried out at 37°C for 2h, an appropriate control being performed. The reaction was terminated by addition of 1.0ml of 10% (w/v) trichloroacetic acid and ninhydrin-positive materials

liberated in the supernatant were determined by the method of Alberti & Bartley (1969). Proteinase activity was expressed as nmol of amino acids released/h per mg of protein. Mitochondria used in this assay were given a prior treatment with a low concentration of digitonin, to remove contaminating lysosomes (Loewenstein *et al.*, 1970).

Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard.

Results

Turnover of mitochondrial proteins

The turnover of whole mitochondrial proteins of liver, kidney and brain is shown in Fig. 1. It is evident that in normal as well as thyroidectomized animals, liver, kidney and brain mitochondrial proteins turn over with first-order kinetics (Fletcher & Sanadi, 1961). The half-lives of liver and kidney mitochondria increase by about 42–45% on thyroidectomy, whereas the corresponding increase seen in the brain mitochondria (Table 1) is relatively negligible.

The observed increase in the half-lives of liver and kidney mitochondrial proteins could be due to some or all protein components. A similar possibility may also exist for brain mitochondria, although no appreciable change is seen in the half-life of these organelles after thyroidectomy. To ascertain this possibility, the mitochondrial proteins were fractionated into several protein components such as water-soluble proteins, cytochrome *c*-rich fraction, contractile proteins, structural proteins and 'other cytochromes' (Beattie *et al.*, 1966) and their turnover rates were examined.

The results in Table 2 show that in the normal animals, all the protein components of liver mitochondria turn over at identical rates, with a mean half-life of about 8.5 days. These observations are consistent with those of Beattie *et al.* (1967). The turnover rates of different protein components of liver mitochondria from thyroidectomized animals, however, are not identical; whole mitochondria, contractile proteins, structural proteins and 'other

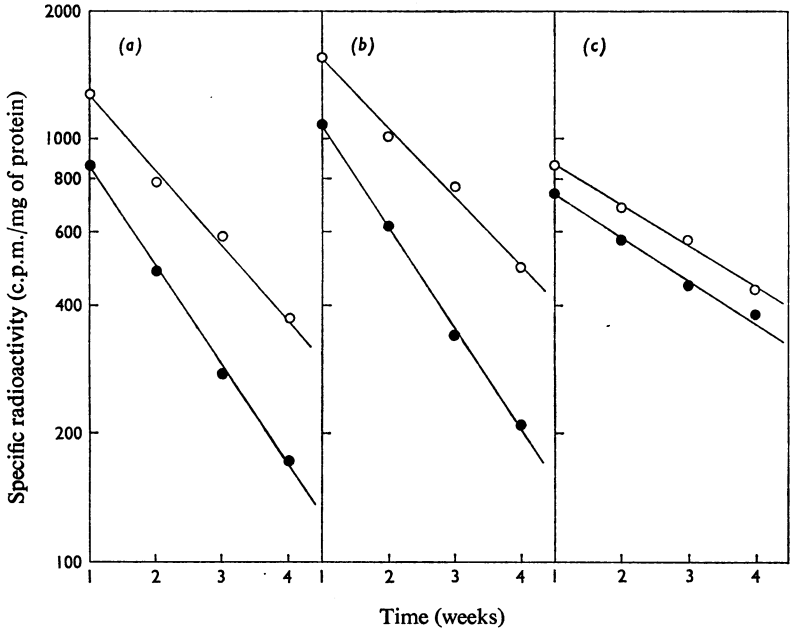

Fig. 1. *Turnover of (a) liver, (b) kidney and (c) brain mitochondrial proteins*

Normal and thyroidectomized rats received 25 μCi of DL-[1-^{14}C]leucine (sp. radioactivity 47.9 mCi/mmol)/100 g body wt. in 0.9% NaCl. The label was allowed to stabilize for 1 week and the rats were killed at the end of 1, 2, 3 or 4 weeks. Mitochondria from liver, kidney and brain were isolated as described in the Materials and Methods section and washed three times. Radioactivity in mitochondrial proteins were counted in a Beckman LS-100 liquid-scintillation spectrometer as described in the Materials and Methods section. The regression of radioactivity in mitochondrial proteins is plotted against time on a semi-log scale. Each point represents the mean of at least four independent experiments. ●——●, Normal; ○——○, thyroidectomized.

Table 1. *Turnover of whole mitochondrial proteins in normal and thyroidectomized rats*

The values of the first-order reaction constant k and $t_{\frac{1}{2}}$ were calculated as described by Fletcher & Sanadi (1961) from data of Fig. 1. Other details are as described for Fig. 1 and in the Materials and Methods section.

Animals	Tissue	First-order reaction constant, k	Standard error of k	$t_{\frac{1}{2}}$ (days)	Increase in $t_{\frac{1}{2}}$ on thyroidectomy (%)
Normal	Liver	0.08365	0.00898	8.28	
	Kidney	0.08081	0.00148	8.57	
	Brain	0.03560	0.00156	19.46	
Thyroidectomized	Liver	0.05777	0.00285	11.99	44.8
	Kidney	0.05652	0.00111	12.26	42.0
	Brain	0.03112	0.00124	22.26	10.2

Table 2. *Effect of thyroidectomy on turnover rates of liver mitochondrial proteins*

Mitochondrial protein components were extracted by the procedure described by Beattie *et al.* (1966), and the radioactivity in the protein samples was counted. The first-order reaction constant k and $t_{\frac{1}{2}}$ were calculated as described by Fletcher & Sanadi (1961). Other details are as in Fig. 1 and Table 1.

Animals	Mitochondrial fractions	First-order reaction constant, k	Standard error of k	$t_{\frac{1}{2}}$ (days)	Increase in $t_{\frac{1}{2}}$ on thyroidectomy (%)
Normal	Whole mitochondria	0.08365	0.00898	8.28	
	Water-soluble proteins	0.08693	0.00519	7.97	
	Cytochrome *c*	0.08752	0.00166	7.92	
	Contractile proteins	0.08429	0.00351	8.22	
	Structural proteins	0.07911	0.00111	8.96	
	'Other cytochromes'	0.07822	0.00640	8.86	
Thyroidectomized	Whole mitochondria	0.05777	0.00285	11.99	44.8
	Water-soluble proteins	0.08367	0.00910	8.02	—
	Cytochrome *c*	0.07416	0.00637	9.34	15.2
	Contractile proteins	0.06639	0.00143	10.43	27.0
	Structural proteins	0.05727	0.00093	12.10	35.0
	'Other cytochromes'	0.06336	0.00275	10.93	36.5

cytochromes' turn over at a lower rate, whereas the water-soluble proteins and cytochrome *c*-containing components turn over at a relatively higher rate. It is apparent that the half-lives of most of the protein components, including the whole mitochondria, contractile proteins, structural proteins and 'other cytochromes', increase to various extents from 27 to 45% after thyroidectomy, whereas the half-lives of water-soluble proteins and cytochrome *c* components are not affected appreciably under this condition.

The protein components of kidney mitochondria from normal animals, like those of liver, turn over synchronously (Table 3). In contrast with the observations of Beattie *et al.* (1967), we did not observe a higher turnover rate for KCl-soluble proteins. The reason for the observed difference is not clear. In kidney mitochondria from thyroidectomized rats, the water-soluble fraction turns over at a higher rate than other protein components (Table 3). The half-lives of whole mitochondria, cytochrome *c*, contractile proteins and structural proteins increased by 30–55% after thyroidectomy, whereas the half-lives of water-soluble proteins and 'other cytochromes' fractions are altered less significantly (12–14%). The effect of thyroidectomy on the turnover of proteins in the kidney mitochondria is thus different from that on the turnover of liver mitochondrial proteins.

The turnover of proteins of brain mitochondria from normal and thyroidectomized animals is shown in Table 4. In the normal animals, all the protein components do not turn over at the same rates, water-soluble proteins and contractile proteins turning over somewhat faster than whole mitochondria. On the other hand, the structural proteins have a somewhat slower turnover. It was not possible to isolate the 'other cytochromes' fraction in sufficient quantities from the brain mitochondria. The turnover studies of this protein component are therefore not included. In brain mitochondria of thyroidectomized animals also, there is no synchrony of turnover in the various

Table 3. *Effect of thyroidectomy on turnover rates of kidney mitochondrial proteins*

Experimental conditions were as described for Table 2. Other details are as given for Fig. 1 and Table 1.

Animals	Mitochondrial fractions	First-order reaction constant, k	Standard error of k	$t_{\frac{1}{2}}$ (days)	Increase in $t_{\frac{1}{2}}$ on thyroidectomy (%)
Normal	Whole mitochondria	0.08081	0.00148	8.57	
	Water-soluble proteins	0.08952	—	7.74	
	Cytochrome *c*	0.08417	0.00183	8.23	
	Contractile proteins	0.07947	0.00412	8.72	
	Structural proteins	0.07277	0.00427	9.52	
	'Other cytochromes'	0.07573	0.01021	9.15	
Thyroidectomized	Whole mitochondria	0.05652	0.00111	12.26	42.0
	Water-soluble proteins	0.08051	0.00532	8.61	11.7
	Cytochrome *c*	0.06019	0.00159	11.52	40.0
	Contractile proteins	0.05122	0.00479	13.53	55.2
	Structural proteins	0.05600	0.00583	12.37	29.9
	'Other cytochromes'	0.06630	0.00241	10.45	14.2

Table 4. *Effect of thyroidectomy on turnover of brain mitochondrial proteins*

Experimental conditions and other details are as in Table 2. Other details were as described for Fig. 1 and Table 1 and the Materials and Methods section.

Animals	Mitochondrial fractions	First-order reaction constant, k	Standard error of k	$t_{\frac{1}{2}}$ (days)	Increase in $t_{\frac{1}{2}}$ on thyroidectomy (%)
Normal	Whole mitochondria	0.03560	0.00156	19.46	
	Water-soluble proteins	0.04677	0.00051	14.81	
	Cytochrome *c*	0.03300	0.00449	21.00	
	Contractile proteins	0.04475	0.00189	15.48	
	Structural proteins	0.02842	0.00402	24.39	
Thyroidectomized	Whole mitochondria	0.03112	0.00124	22.26	10.2
	Water-soluble proteins	0.03964	0.00204	17.48	18.0
	Cytochrome *c*	0.05269	0.00754	13.15	−37.4
	Contractile proteins	0.03277	0.00115	21.14	36.7
	Structural proteins	0.02494	0.00065	29.73	21.9

protein components, the water-soluble proteins and cytochrome *c* turning over at a higher rate than the other proteins. A comparison of the two groups indicates that, although thyroidectomy apparently does not affect the half-life of brain mitochondria as a whole to any appreciable extent, it causes a rapid turnover of the cytochrome *c* fraction, the decrease in its half-life being about 37%. The half-lives of water-soluble proteins, structural proteins and contractile proteins increased by 18–37% compared with the control.

Turnover of mitochondrial DNA

Table 5 shows the data on half-lives of liver and kidney m-DNA (mitochondrial DNA) from normal and thyroidectomized rats. In normal animals, the values for half-lives of m-DNA from liver and kidney are comparable with the half-lives of the respective mitochondria. This is in agreement with the observations of other workers (Gross *et al.*, 1969). On thyroidectomy, however, their half-lives increase by about 44%, a value comparable with the increase in the half-life of the whole mitochondria (Table 1) and also of insoluble proteins (Tables 2 and 3). DNA could not be obtained from brain mitochondria in sufficient quantities. Besides, the incorporation was also low. Hence data on this are not included. Similar difficulty has also been encountered by other workers (Gross *et al.*, 1969).

Determination of mitochondrial protein components

The data in Table 6 show the quantitative distribution of water-soluble proteins, cytochrome *c* and the contractile proteins. The 'residue' represented

Table 5. *Effect of thyroidectomy on the turnover of mitochondrial DNA*

Normal and thyroidectomized rats received two doses of 100μCi of [^{3}H]thymidine/100g body wt. in 0.9% NaCl on consecutive days and were killed on days 1, 7, 15 or 21 after the second injection. Mitochondria were incubated with DNAase I to remove extraneous DNA, as described in the Materials and Methods section (Gross *et al.*, 1969). m-DNA was obtained by the procedure of Parsons & Simpson (1973) and the radioactivity was counted as described in the text. Each point was a mean of three independent experiments. As described in the Results section, brain m-DNA could not be obtained in sufficient quantity: also, DNA showed low incorporation, hence data on its half-life are not included.

Tissue	$t_{\frac{1}{2}}$ of mitochondrial DNA (days)		Increase in $t_{\frac{1}{2}}$ on thyroidectomy (%)
	Normal	Thyroidectomized	
Liver	7.30	10.50	44.0
Kidney	7.44	10.79	44.1
Brain	—	—	—

Table 6. *Recovery of mitochondrial protein components from liver, kidney and brain of normal and thyroidectomized rats*

Mitochondrial protein components were isolated as described by Beattie *et al.* (1966). Since it was not possible to recover quantitatively structural proteins and 'other cytochromes' the value for total protein comprising these two protein components is represented as 'Residue'. The values are given as mean±S.E.M. of at least four independent experiments.

Tissue	Protein components	% of total mitochondrial protein in	
		Normal	Thyroidectomized
Liver	Water-soluble proteins	10.48±0.82	13.75±0.55
	Cytochrome *c*	3.00±0.22	3.42±0.26
	Contractile proteins	3.38±0.19	5.53±0.32
	'Residue'	63.20±2.64	58.00±2.32
	Recovery	80.06	80.70
Kidney	Water-soluble proteins	8.20±0.41	8.52±0.29
	Cytochrome *c*	3.45±0.15	5.49±0.28
	Contractile proteins	2.80±0.11	7.23±0.29
	'Residue'	81.12±4.02	68.34±3.65
	Recovery	95.57	89.58
Brain	Water-soluble proteins	21.68±0.83	21.95±0.64
	Cytochrome *c*	7.16±0.48	5.05±0.27
	Contractile proteins	5.28±0.46	5.28±0.25
	'Residue'	49.40±2.44	42.24±1.91
	Recovery	83.52	74.52

structural proteins and 'other cytochromes', as it was not possible to recover these two quantitatively. The relative proportion of the contractile proteins in liver mitochondria increases to a considerable extent after thyroidectomy. Similarly, in kidney mitochondria, there is a significant increase in the cytochrome *c* and contractile-protein fractions. In brain mitochondria, the cytochrome *c* fraction decreases, although no changes were seen in the contractile proteins. It is thus evident that quantification of the relative proportions of the different protein components in normal and thyroidectomized rats correlates fairly well with the observed changes in the half-lives after thyroidectomy, although a strict parallelism could not be drawn. These results thus indicate that the specificity of action of thyroid hormones is different for different tissues. The mitochondrial protein content of the tissues, however, remained unaltered under these conditions (Table 7).

Lysosomal cathepsin activities

Lysosomes have often been implicated in the degradation of mitochondria (Ashford & Porter, 1962; Tappel *et al.*, 1963; Swift & Hruban, 1964). In view of this, it was decided to examine the cathepsin activities in the different tissues of normal and thyroidectomized rats (Table 8). Kidneys contain a greater amount of cathepsin activity than does liver, although the half-lives of kidney and liver mitochondria are comparable in the normal euthyroid state (Table 1). Brain shows lower cathepsin activity compared with liver. Thyroidectomy, however, did not affect the cathepsin activity significantly, although, as described above the turnover rates of liver and kidney mitochondria are affected to an appreciable extent (Table 1). These results therefore indicate that probably lysosomal cathepsins are not involved in the degradation of mitochondrial proteins.

Table 7. *Effect of thyroidectomy on mitochondrial protein content in liver, kidney and brain*

Mitochondrial protein content of the tissues was determined on the basis of succinate dehydrogenase activities of homogenates and three-times-washed mitochondria from the corresponding tissues (Gross, 1971). Values are given as mean±S.E.M. of at least six independent experiments.

Tissue	Mitochondrial protein (mg/g wet wt. of tissue)	
	Normal	Thyroidectomized
Liver	50.59±3.44	57.20±5.05
Kidney	64.01±8.96	66.99±3.46
Brain	33.51±4.12	28.09±3.06

Table 8. *Cathepsin activities in liver, kidney and brain of normal and thyroidectomized rats*

Cathepsin activities were determined by using a 10% tissue homogenate as the enzyme source. 'Free' activity represents the cathepsin activity when the homogenate was not subjected to freezing and thawing. For determination of 'Total' activity, the homogenate was subjected to freezing and thawing six times. A small amount of Triton X-100 was also included in the assay medium. Assay of cathepsin activity was as described by Gianetto & de Duve (1955). Values are given as mean±S.E.M. of at least six independent experiments.

		Specific activity (μg of tyrosine released/10min per mg of protein)	
Animals	Tissue	Free	Total
Normal	Liver	0.634±0.066	2.956±0.291
	Kidney	3.041±0.314	5.514±0.239
	Brain	0.576±0.062	2.138±0.235
Thyroidectomized	Liver	0.752±0.117	2.777±0.259
	Kidney	2.408±0.084	6.134±0.380
	Brain	0.668±0.052	2.511±0.237

Mitochondrial proteinase activities

In view of the report from this laboratory suggesting the possibility of the involvement of the mitochondrial proteolytic enzyme in the degradation of mitochondrial proteins (Subramanian, 1975), we undertook a study of this enzyme in different tissues of the normal and thyroidectomized animals. Table 9 shows that liver and kidney mitochondria have comparable proteinase activities, whereas the brain mitochondria have very low activity. This becomes more evident in sonicated samples, where all of the enzyme activity is exposed. Also, the proteinase activities in mitochondria show a fairly good correlation with their respective half-lives. In thyroidectomized rats, this activity decreases by 28–53% in kidney and liver mitochondria, consistent with the observed decrease in the half-lives of these organelles, brain mitochondria showing a marginal decrease of about 14%. These results are indicative of a possible involvement of this enzyme in regulating the turnover of the mitochondrial proteins.

Discussion

Thyroidectomy and mitochondrial protein turnover

Our present studies with normal animals show results similar to those of Beattie *et al.* (1967). The mitochondrial proteins of liver as well as of kidney turn over at rates almost identical with those for the respective mitochondria. Thus, in agreement with the original proposal of Fletcher & Sanadi (1961), in normal rats the liver and kidney mitochondria seem to turn over as an entity. For brain mitochondria, we have extended our studies to examine in detail the turnover rates of cytochrome *c*, contractile proteins and structural proteins; Beattie *et al.* (1967) have examined the turnover only of water-soluble and -insoluble proteins. As shown in Table 4, the water-soluble as well as the contractile proteins turn over at a relatively higher rate, thus emphasizing the asynchronous turnover of brain mitochondrial proteins even in the euthyroid state.

The results with thyroidectomized animals indicate that the synchrony of turnover of liver and kidney mitochondrial proteins in the euthyroid state is lost after thyroidectomy (Tables 2 and 3). Thus in liver and kidney mitochondria, cytochrome *c*, contractile proteins, structural proteins and 'other cytochromes' fractions turn over at a slower rate. The extent to which these turnover rates are affected is variable. Practically the same thing holds true for brain mitochondria, except for the cytochrome *c* component, which turns over at a relatively higher rate. These results, besides indicating the regulatory role of thyroid hormone(s) in maintaining the synchrony of turnover of liver and kidney mitochondria, also point to a tissue-specific regulatory role of the hormone(s). It is noteworthy in this connexion that Gustafsson *et al.* (1965) observed that when chronic thyroid-hormone treatment is withdrawn, the enzyme activities decrease, but the increased cristal structures of skeletal-muscle mitochondria disappear at a much later stage. These observations are in agreement with our present studies on asynchronous turnover after thyroidectomy.

Tables 2, 3 and 5 show that the turnover rates of m-DNA from liver and kidney coincide well with that of whole mitochondria in normal as well as in thyroidectomized rats. Thyroidectomy results in a corresponding increase in the half-lives of these m-DNA species. These results are in agreement with those of other workers (Gross, 1971). The increase in the half-lives of DNA paralleled the increase in the half-lives of contractile proteins and structural proteins of these organelles (Tables 2, 3 and 5). This is notable in view of the fact that structural proteins represent the insoluble membrane proteins and contain the gene products of m-DNA (Roodyn & Wilkie, 1968). These observations therefore lend support to the suggestion of Gross (1971) that 'the mitochondrial turnover can be predicted upon a large subunit containing much of the protein and DNA,' and also the suggestion by Gear (1970) of a probable turnover of inner membrane and matrix surrounded by a primary outer membrane turning over as a unit.

In the present studies, the label used to examine the turnover rates was [1-^{14}C]leucine. The problem of label re-utilization is therefore encountered. However,

the fact that in thyroidectomized rats the rates of turnover of different proteins in the various organs are affected to various extents indicates a differential effect of thyroidectomy. This cannot be explained only on the basis of label re-utilization. The regulatory role of thyroid hormone(s) is therefore obvious. Menzies & Gold (1971) studied the turnover of mitochondrial proteins by using [^{3}H]leucine in young adults and aged rats and believe that re-utilization is not a major factor in determining the turnover constant. They have discussed this in detail and forwarded several reasons for such an assumption (Menzies & Gold, 1971). Hence it seems likely that the tissue-specific effect that we observe truly relates to the regulatory role of thyroid hormone(s). Our earlier results on protein synthesis *in vivo* and *in vitro* also support such an assumption (Katyare *et al.*, 1970; Satav *et al.*, 1973).

Thyroidectomy and mitochondrial protein composition and content

The compositional changes observed in mitochondrial components (Table 6) correlate fairly well with the observed turnover rates. However, one has to consider the possibility that, besides relating to the turnover rates, the compositional changes may also reflect changes brought about as a result of thyroidectomy itself.

The data of Table 7 show that the mitochondrial protein content of the three organs does not change significantly as a result of thyroidectomy. These probably represent the deficient mitochondria or are products of a compensatory mechanism (Gustafson *et al.*, 1965). Indeed our other studies have shown that in thyroidectomized animals, rates of mitochondrial oxidation decreased significantly in all the three tissues under study (M. S. Rajwade, S. S. Katyare, P. Fatterpaker & A. Sreenivasan, unpublished work).

Cathepsin and mitochondrial proteinase activities in relation to mitochondrial turnover

Involvement of lysosomes in mitochondrial turnover has been suggested by several workers (Ashford & Porter, 1962; Tappel *et al.*, 1963; Swift & Hruban, 1964). Swift & Hruban (1964) observed, by electron microscopy, mitochondria in various stages of degradation inside lysosomes of rat pancreas, liver and prostate gland. Administration of toxic factors increased the number of such incidents. On incubation with lysosomes *in vitro*, mitochondria are known to be digested (Tappel *et al.*, 1963). On the other hand, in the regressing tail muscle of *Xenopus* larvae, during metamorphosis, autolysis is observed in the absence of lysosomal enzymes (Weber, 1964). Novikoff (1963) studied regression of lymphocytes in thymus after cortisone treatment or X-irradiation and observed profound changes in mitochondria and cytoplasm without marked activity of lysosomes. Another example is of increased proteolysis in the presence of β-glycerophosphate, which gives protection to lysosomes (Alberti & Bartley, 1969). Our results in Table 8 show that there is no correlation between the cathepsin activity and rate of mitochondrial turnover in any of the tissues studied. Moreover, although thyroidectomy decreased the rate of mitochondrial turnover, the lysosomal cathepsin activity in these tissues remained practically unaltered. It therefore seems that cathepsins are probably not responsible for the degradation of mitochondria under normal physiological conditions, although this seems to be the case in pathological conditions (Swift & Hruban, 1964). The observation that mitochondrial proteins in liver increase after X-irradiation, whereas the free lysosomal activity increases at the expense of bound activity (K. C. Alexander, A. S. Aiyar & A. Sreenivasan, unpublished work), is also consistent with such an assumption.

Table 9. *Effect of thyroidectomy on mitochondrial proteinase activity*

Mitochondrial proteinase activity was determined as described in the text. Sonication of mitochondria was carried out in a MSE sonicator at 1.5kHz for 2min at 0–4°C. Results are given as mean±S.E.M. of at least eight independent experiments.

Animals	Tissue	Specific activity (μg of leucine/h per mg of mitochondrial protein)		Decrease on thyroidectomy in sonicated samples (%)
		Unsonicated	Sonicated	
Normal	Liver	23.81±2.79	32.56±4.71	
	Kidney	18.59±0.75	31.36±3.57	
	Brain	5.81±0.51	8.45±0.48	
Thyroidectomized	Liver	13.63±1.39	15.44±1.80	53
	Kidney	16.85±2.29	22.89±2.24	28
	Brain	4.82±0.51	7.20±0.61	14

On the other hand, mitochondria by themselves have enough proteinase activity (Table 9). This activity increases only slightly on sonication (Table 9), unlike in the case of lysosomes. This would probably mean that the enzyme activity is always accessible even within the intact mitochondria and sonication is not necessary to expose the complete activity. Also, the enzyme activity correlates well with the half-lives of the mitochondria from different tissues in normal as well as thyroidectomized rats (Table 9). Hence it seems likely that this enzyme may be involved in the degradation of mitochondrial proteins.

Mitochondrial enzymes such as ornithine aminotransferase, alanine aminotransferase and δ-aminolaevulinate synthetase, which have fairly short half-lives, are inserted and removed from mitochondria independent of synthesis and destruction of the entire organelle (Swick *et al.*, 1968; Marver *et al.*, 1966). It seems likely that mitochondrial proteinases may play an important role in regulating such enzyme contents. Such an assumption is supported from other findings from our laboratory that this enzyme is specific for mitochondrial proteins such as cytochrome *c*, structural protein etc., and that denatured mitochondrial proteins serve as better substrates (Subramanian, 1975). This enzyme has a different pH optimum from the cathepsins and, unlike cathepsins which act non-discriminately on all cellular proteins, this enzyme acts only on mitochondrial substrates as described above. The end product of the enzyme reaction is also different. Cathepsins give tyrosine-containing peptides, whereas the products of mitochondrial proteinase activity are tyrosine-negative and the enzyme activity could be detected only by the ninhydrin assay. These differential properties and its specificity towards mitochondrial substrates make its role in mitochondrial turnover more relevant (Subramanian, 1975).

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